ON THE REMOVAL OF DIFFUSIBLE SUBSTANCES FROM THE CIRCULATING BLOOD OF LIVING ANIMALS BY DIALYSIS

BY

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From the Pharmacological Laboratory of the Johns Hopkins University

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I. INTRODUCTORY

There are numerous constituents of the blood derived from various organs and of vital significance to the economy, and present in the blood in only minute quantity at any one time, which can not be isolated or identified by the means now at our command. When the proteids of the blood, for example, are removed by precipitation in the course of an analysis, the minute quantities of the substances in question are carried down with them and adhere firmly to them. Nor do the kidneys or other excretory organs eliminate these substances in quantities that suffice for detection or study.

It is evident that only by some method which will remove from the flowing blood the traces of these substances as fast

as they are poured into it, without at the same time removing proteids or cellular elements, can we accumulate them in sufficient amounts for study.

Again, there are numerous toxic states in which the eliminating organs, more especially the kidneys, are incapable of removing from the body at an adequate rate, either the autochthonous or the foreign substances whose presence in excessive amount is detrimental to life processes. In the hope of providing a substitute in such emergencies, which might tide over a dangerous crisis, as well as for the important information which it might be expected to provide concerning the substances already referred to as normally present in the blood, and also for the light that might thus be thrown on intermediary stages of metabolism, we have devised a method by which the blood of a living animal may be submitted to dialysis outside the body, and again returned to the natural circulation without exposure to air, infection by micro-organisms, or any alteration which would necessarily be prejudicial to life. The process may be appropriately referred to as "vivi-diffusion." The apparatus constitutes what has been called an artificial kidney in the sense that it allows the escape of the diffusible constituents of the blood, but it differs from the natural organ in that it makes no distinction between these constituents, the rate of their elimination being presumably proportional to the coefficients of diffusion. It will be shown, however, that any given constituent of the blood, as urea, sugar or sodium chloride can be retained in the body by a simple expedient when so desired.

Our first demonstration of vividiffusion performed on a rabbit ran successfully for two hours and was given before colleagues in Baltimore on November 10, 1912. On May 6, 1913, we read a paper with the above title before the Association of American Physicians[1] in Washington and during the past summer demonstrations of the method were given by us in London[2] and in Groningen. Various circumstances have combined to hinder us from giving until now the details of our method.

[1] Trans, Assoc. Amer. Physicians, 1913.
[2] British Med. Journ., Aug. 11, 1913.

II. THE METHOD

The principle of the method consists in connecting an artery of the animal by a cannula to an apparatus made of celloidin or other dialyzing membrane, in the form of tubes, immersed in a saline solution or serum,[3] and providing for the return of the blood to the animal's body by another cannula attached to a vein. The tubes and cannulae are filled completely before attachment with a saline solution which approximates in composition to the salt content of the serum of the animal. This is displaced into the body by the inflow of blood, when the circulation in the apparatus is established. The blood leaving the artery flows through a perfectly closed system and returns to the body within a minute or two without having been exposed to contact with the air or any chance of microbial infection, while the diffusible substances which it contains can pass out, more or less rapidly through the walls of the tubes. Coagulation of the blood is prevented by injection of hirudin. The substitution in the animal's body of saline solution for an equal volume of blood leaves the physiological condition as nearly as possible unchanged, and chemical results obtained by this method may be expected to represent normal conditions very closely, closer, e.g., than when large quantities of blood are drawn off for analysis.

The proportion of blood (i.e., after admixture of saline)[4] tem-

[3]The outer fluid may, of course, be water, but this leads very quickly to haemolysis of the red corpuscles. Any constituent of the blood which it is desired to retain in the animal's system may be prevented from dialyzing out by the addition of the proper amount to the outer fluid. Where the object of the experiment is merely to remove from the blood abnormal constituents, as e.g., poisons, or constituents specifically secreted into the blood by a certain organ, normal serum from a similar animal may be used, thus insuring complete balance of all normal constituents, inside and out.

[4]The blood in such cases is, of course, correspondingly diluted by the volume of saline solution originally present in the apparatus, so that, for example, if the volume of a dog's blood be taken at 74 cc. per kilogram, an apparatus holding 37 cc. per kilogram, would, at the beginning of an experiment, hold not one-half but one-third of the total blood corpuscles, serum proteids, etc. But owing to the well known phenomena of the automatic regulation of the concentration of the blood by absorption by the tissues, it is probable that the admixed saline is removed more or less rapidly, thus diminishing the total volume and increasing the proportion outside the body.

porarily outside the body in the dialyzing apparatus may be very high: for short periods a dog will tolerate a dialyzing apparatus holding as much as 40 cc. per kilogram of body weight while one holding 30 cc. may be used for many hours.

When the circulation in the apparatus is established there is a fall in the blood pressure which is greater or less according to the size of the apparatus in proportion to that of the animal, but there are no other immediate symptoms. Rapid and complete recovery after an experiment lasting many hours may be obtained by due regard to asepsis and care in the use of the anaesthetic. Serious loss of blood is avoided by driving the greater part back into the animal's body at the end of the experiment. This may be done either by blowing air, or better by introducing a saline solution from an elevated burette, into the arterial end of the apparatus after tying off the artery, while the venous cannula is still attached: or by a simpler and more rapid method, which returns about three-quarters of the blood, and which in an emergency can be used during the progress of the experiment to relieve the threatened collapse of an animal in an abnormally weak condition; this consists in applying hydrostatic pressure to the liquid outside the tubes (viz., by blowing or forcing more liquid into the jacket which contains it) and thereby squeezing them flat. For purely chemical investigations the experiment is usually performed under complete chloretone anaesthesia and allowed to run as long as the animal lives. Owing to the weakening effects of the operation, the anaesthetic, the constrained position and the loss of substances from the system, the length of dialysis obtained has not usually exceeded eight or ten hours with an apparatus measuring 30 cc. per kilogram of body weight, but in one case a dog survived for sixteen hours with one-third of its blood in the dialyzing apparatus.

III. THE APPARATUS: TYPES AND METHODS OF CONSTRUCTION

The dialyzing substance which appears best adapted to the purpose of the research, and the only one tried at present, is celloidin. The brand which we have used is "Anthony's Nega-

tive Cotton," manufactured by the Ansco Company, Binghamton, N. Y., as this proved to be nearly free from acid reaction. A solution is made of 10 grams of the celloidin in 100 cc. ether and 100 cc. ethyl alcohol: the solid swells up and dissolves with occasional gentle stirring in about 48 hours, but as a small amount of brown sediment separates on standing it is better to allow it to remain three or four days longer and decant the clear upper portion for use. Much time and ingenuity have been expended in attempts to devise a practicable method of constructing a whole system of fine branched tubes at one operation but so many difficulties were encountered that it has been found preferable, up to the present, to make each tube separately and fasten them to each other and to the inflow, outflow and connecting tubes by tying with thread. The most serviceable size of tube has a diameter from 6 mm. to 8 mm. and a length from 20 cm. to 50 cm. These are best made inside glass tubes, which should be about 1 mm. to $1\frac{1}{2}$ mm. larger in internal diameter to allow for shrinkage in manufacture and sufficient clearance in attachment. The smaller sized tubes are simply filled once with the celloidin mixture which is then allowed to drain out while the tube is held in a vertical position, then reversed and fastened in a retort stand. After about 10 to 15 minutes,[5] during which the tubes may be reversed once or twice if there are signs of accumulation of all the fluid at one end, the tube is redipped, first at one end and, after about 5 minutes, again at the other, immersing in each case only a few centimeters, in order to provide a thicker film at the ends for ease and safety in tying. The central portion should be as thin as possible for ease of dialysis, consistent with stiffness sufficient to prevent wrinkling or kinking when handled, which easily produces pin-hole leaks. Larger tubes are best made by rotating the tube in the hands and reversing frequently, to keep the fluid evenly distributed till dry enough not to flow. In all cases a couple of centimeters should be left over at each end for cutting off, to avoid irregularities.

The proper time of evaporation is very important and can be best told by trial. The safest indication is the smell to be noticed

[5] These times will be longer in cold weather.

by applying the nose to the *lower* end of the upright tube. As soon as ether can no longer be detected in this manner, giving place to a smell of almost pure alcohol the whole tube is immersed in water and allowed to soak for about 10 minutes, when the film of celloidin will tend to separate from the glass, having shrunk slightly as the alcohol in it was replaced by water. After peeling off the protruding ends and lifting the edges away from the glass cautiously with a knife or needle point, the whole film can be slid out without using appreciable force. In case of obstinate adhesion, or tubes of irregular diameter, a slender pointed glass rod may first be pushed up between the film and the glass, which will cause the former to peel away quite easily ahead of the end of the rod without danger of puncture. A good film, dipped in water at the proper time, will be soft and pliable as fine kid, yet posessed of considerable tensile strength for steady pulls. Sudden jerks or local pinching easily tears the material, which is seen, when once a tear starts, to be as soft as jelly. A film that has dried too far will be tougher and more glossy, but owing to the shrinkage of the material and reduction of the water content in its interstitial spaces, it will not give such ready dialysis. If dipped in water too soon the film will be deficient in strength, and if much ether was left, it will turn cloudy in water, owing to the precipitation of the celloidin which was still dissolved at the time of immersion. The thickness of the film, according to preliminary experiments, appears in practice to have no appreciable influence on the speed of diffusion of substances through it, within the limits tested. This unexpected result can only be explained on the theory that owing to stagnation of a layer of fluid of considerable thickness on either side of the film the resistance to diffusion in the film itself is only a small part of the total resistance. The films used measure in their central portion about 0.05 mm. to 0.1 mm. in thickness in the moist state, the thickened ends being about 0.2 mm. Each gram of celloidin will make about 400 sq. cms. of diffusing surface, disregarding losses in manufacture.

The dialyzing tubes are attached at the ends to glass tubes of like diameter and the whole is contained in a glass jacket, like a

Liebig's condenser, provided with an inlet and outlet for the outer fluid.

The best form of apparatus has been the subject of much thought and experiment. Maximum diffusing surface combined with as small a volume as possible is the chief point in view. This can be attained either by using many very small tubes, or by flattening a larger tube till the opposite surfaces nearly touch. The former method is limited by the complexity of the necessary connections, the time required in making and attaching the celloidin tubes, and the difficulty of tying absolutely secure knots in very narrow spaces between a great number of tubes; as well as by the increasing chance of an accidental leak which may necessitate the disconnection of a large part of the apparatus. The latter method seems to promise good results, but owing to a number of inherent difficulties which will be discussed later with the description of the apparatus, it is still in process of trial.

The tubular type of apparatus for an animal of moderate size, like a dog, may have from about sixteen tubes of the size described above, up to two or three times that number. Uniformity of flow in all tubes, which is a very important consideration, is most easily attained where the number of tubes is a power of two, as in that case the distribution of the flow can be effected by successive dichotomous branching; but in any case great care must be taken that all branching channels are as nearly as possible of the same width, length and directness, while all sharp angles and sudden bends must be avoided.

Another important consideration is to keep down to a minimum the length of all glass connections and the area of surface of glass in contact with blood, as the alkali which is taken up from this surface tends to induce clotting.[6]

The original apparatus (of only four tubes, used on a rabbit) was attached to the carotid artery and femoral vein, being approximately the same length as the animal's body.

A great improvement in practice was effected when the course

[6] Coating the inside of the tubes and the connecting cannulae with paraffin has been tried but discarded, owing to obstruction by flakes which become detached during the course of an experiment.

of the blood in the apparatus was made to return on itself after the manner of a U-tube, so that inflow and outflow tubes were at the same end and close together. This arrangement, which has never since been abandoned, allows of attachment by short cannulae, e.g., to the carotid artery and vein, or femoral artery and vein of the same, or usually of opposite sides. In an apparatus of this type, two or three forms of which are described below, the glass parts consist of branching inflow and outflow tubes at the proximal end, and branching tubes connected by a U shaped bend at the distal end and in some cases where the blood is intended to flow twice or more times each way in the apparatus at the proximal end also.

For ease in tying of the celloidin tubes, and avoiding the chance of displacement, wrinkling, or kinking, with the formation of leaks, it is essential to have the two glass tubes to which a celloidin tube is to be tied at each end, rigidly connected by a framework, preferably independent of the outer jacket or case. A central glass rod, or frame-work of two or more lateral rods is therefore used as a skeleton connecting the glass tubes at *each* end with each other and with the cork or stopper at the proximal end, into which it is fastened, so that the whole can be lifted out by this one cork: the celloidin tubes are then tied on in the open, where everything is accessible, and the finished system put back into the jacket, which is closed by a separate cork at the other end. The glass rod or rods may be fused to the tubes when made, at the blow-pipe, or, as is found preferable for the more complex systems, connected through the means of the rubber cork at one end, and a corresponding but smaller rubber block at the other. This provides a little elasticity, which makes for ease in tying the inner tubes, and reduces the chance of breakage of glass parts or tearing of tubes by unequal strains. A further not inconsiderable advantage is that it allows of tightening up all the tubes slightly, after tying, by gently sliding the rubber block on the rods. The shape of the branching tubes is designed so as to combine the uniform distribution of flow already referred to, with the shortest possible course on the one hand and a good utilization of the available space in the cross-section

of the jacket, on the other. Diffusion is favored moreover, by spacing the tubes as uniformly as possible, so that there are no narrow stagnant spaces between certain tubes, the liquid in which would soon become saturated with the diffusing substances. On the other hand, too widely separated tubes would require a very large jacket containing an inordinately large volume of liquid, with a correspondingly weak diffusate. Good results are obtained when the nearest distance between two tubes is equal to, or but little less than, their diameters, as in the following cases.

The flow of blood, when divided between too many wide tubes, connected "in parallel," will be so slow in each tube that stagnation, and consequent separation of the corpuscles, which settle along the lower sides of the tubes, may occur as soon as the animal's heart-beat becomes weaker. This is bad for diffusion, and may tend to favor the formation of clots, with possibly other deleterious changes in the composition of the blood. Eight tubes of 8 mm. diameter are as many as can be used in parallel with advantage in the case of a large dog (40 lb. to 50 lb.), though for larger animals with a greater pulse-volume more might be used; but experience would indicate the advisability of keeping somewhat below this number. Too narrow and too long tubes, connected "in series," so that the blood has a very long course before returning to the body, would mean a higher hydrodynamic resistance, but compared with that in the microscopic capillaries of the body, this is usually so low that there does not appear to be much danger of going too far in this direction.

The time that a given portion of blood stays in the apparatus is an important factor in respect of the danger of clotting or undergoing possible deleterious changes. This depends, however, solely on the relation of the total internal volume of the system of tubes to the volume per minute flowing in from the artery. The manner of dividing the stream and course of the flow have no influence on it, as might be mistakenly supposed, since a longer course necessarily means a narrower channel and, therefore, a greater velocity in that channel, the one influence exactly balancing the other.

The apparatus shown in figure 1 has sixteen celloidin tubes of 8 mm. diameter and 20 cm. length of free surface. The arterial cannula *A* is connected by a rubber tube to the inflow tube *D* which bifurcates in a vertical plane at *P* just beyond the rubber stopper. The blood in each channel again divides into four equal streams at *Q* and *R* respectively. The blood thus flows through eight tubes "in parallel" to the further end of the apparatus returning by eight tubes on the other side. The glass rod through the centre of the rubber stopper at *F* forms the skeleton of the apparatus, branching in a vertical plane at the further end at *G* to the points *H* and *K*, where it serves to support the two systems of glass tubes to which the further ends of the celloidin tubes are tied. These are similar in shape and each consists of a U-tube in a horizontal plane with quadruple branches at each end similar to those at *Q* and *R*. The supporting rods are fused onto the walls of the U-tubes at the center of each bend. By this means the blood in four tubes is collected into one (viz., at *K*) as shown by the arrows and again distributed to four tubes on the further side. The outflow tube, which is exactly similar to the inflow tube, collects the blood from the eight return-flow tubes, and is connected at *E* by rubber tubing to the venous cannula *B*. A thermometer passes through the cork at *T*.

The outer liquid is introduced and withdrawn through the glass tube *M* which runs along the bottom of the apparatus to the end nearest the animal, while the short tube at *O* serves to let the air in or out as the case may be. Figure 2 shows the cross section of the sixteen tubes with their connections: the letters refer to the same points as before. The jacket in this apparatus has a length of about 36 cm. and a diameter of 8.5 cm., which allows of spacing the nearest tubes about 16 mm. apart from center to center, or 8 mm. between adjacent sides. The central rod is 8 mm. diameter.

The arterial cannulae used in all these experiments are constructed with a side tube terminating in a fine glass jet which is fused in at the bend as shown in figure 3. This tube (*C* in figs. 1 and 3) is connected by a narrow rubber tube to a burette (*L*, fig. 1) and is used for the injection of hirudin solution, which,

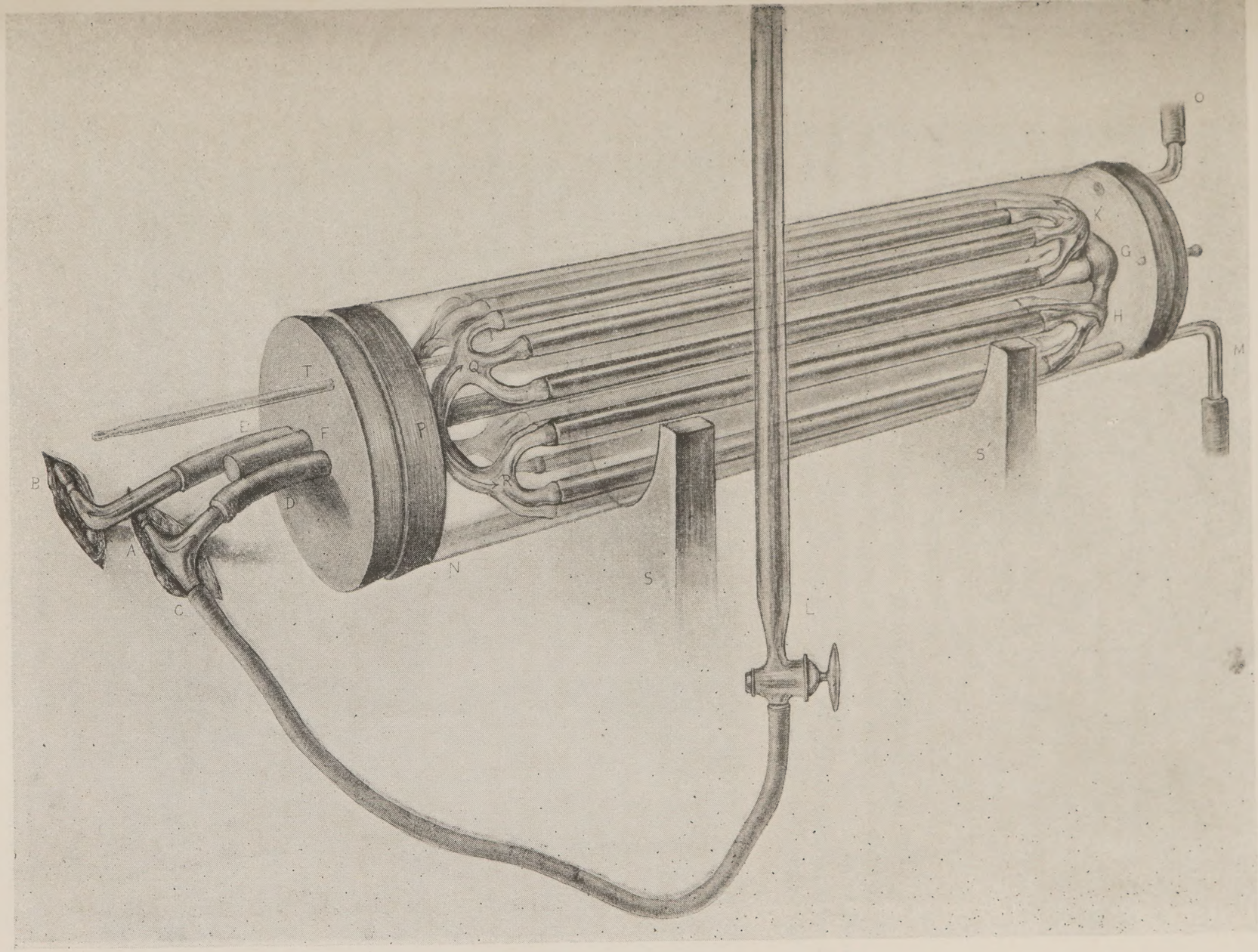

FIG. 1 PERSPECTIVE VIEW OF VIVIDIFFUSION APPARATUS; EARLIER FORM WITH SIXTEEN TUBES

A, arterial cannula; *B*, venous cannula; *C*, side tube for introduction of hirudin; *D*, inflow tube; *E*, outlet tube; *F*, *G*, supporting rod attached at *H* and *K* to branched U-tubes; *L*, burette for hirudin; *M*, *N*, tube for filling and emptying liquid in outer jacket; *O*, air outlet; *P*, dichotomous branching point of inflow tube; *Q* and *R*, quadruple branching points of same; *S*, *S'*, wooden supports; *T*, thermometer. At each of the points *H* and *K* the blood is collected from four tubes into one, bending around to the back, and there redividing into four return flow tubes. Arrows show the direction of flow.

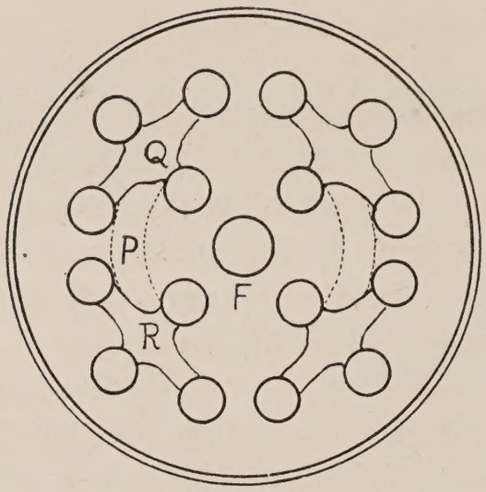

FIG. 2. CROSS SECTION OF APPARATUS SHOWN IN FIGURE 1

F, central supporting rod; *P*, point of dichotomous branching of system of blood tubes; *Q* and *R*, quadruple branch points. The sixteen small circles show the arrangement of the celloidin tubes; the blood flows up left-hand eight and down in the remainder.

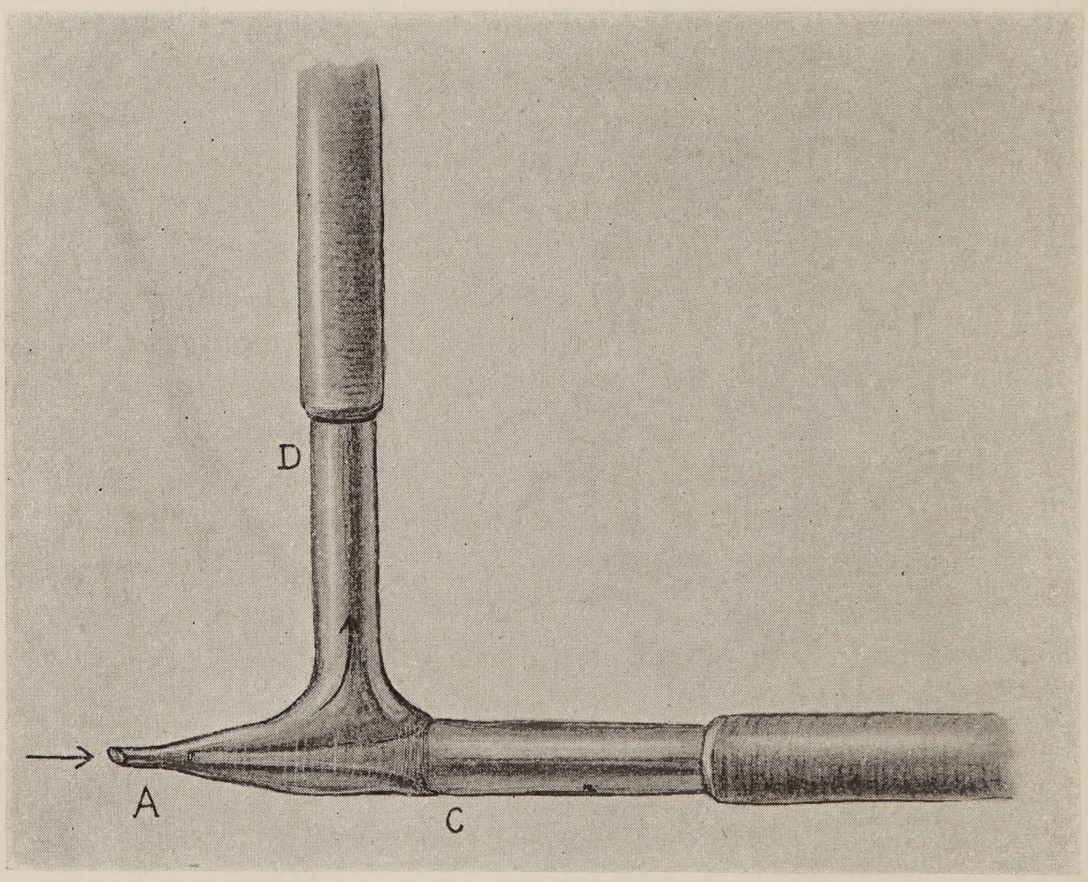

FIG. 3. ARTERIAL CANNULA

C, point at which side tube and inner jet for hirudin are fused in; *D*, end connected by rubber tube to vividiffusion apparatus. The arrows show the direction of blood flow.

being delivered by the jet close up inside the point of the cannula prevents the formation of a clot at that point. The same arrangement serves for the introduction of other substances when desired. For attachment to the portal vein cannulae of the form shown in figure 4 are used. Here *A* and *B* indicate the open ends inserted into the distal end of the portal vein and the proximal end of the same or into the vena cava respectively; *C* and *D* are the ends to be attached to the apparatus, while *E* is the hirudin tube, the jet of which is fused in at *F; G* is a short glass rod fused to the walls of the hirudin and blood tubes to hold them together and support them firmly. This is found to be a useful precaution to prevent breakage at the fragile joint *F*. The cannulae are made with long vertical stems to allow of reaching the bottom of the abdominal cavity.

For large dogs an apparatus is used which has 32 celloidin tubes of 8 mm. diameter and 40 cm. length of free surface contained in a jacket 10 cm. diameter by 53 cm. long. The plan of the tubes is shown in figure 5. In each quadrant are eight tubes connected together, through which the blood flows in parallel, while the course is four times the length of the apparatus, twice up and twice down, the passage from quadrant to quadrant being provided by three-branched U-tubes, two at the distal and one at the proximal end. The framework consists of two glass rods (*S*, *S'* in figs. 5, *D* and *E* in fig. 6) fastened in the cork at the proximal end (a view of which from the outside is seen in fig. 6) and carrying at the further end a rubber block or cross-piece with recesses into which the U-tubes are strapped. In figure 6, *F* represents the thermometer (*T* in fig. 5) while *C* represents the end of a glass rod fused to and supporting the branched U-tube connecting the two upper quadrants: *A* and *B* are the tubes for the inflow and outflow of blood and *G* is an extra hole leading into the jacket.

The arrangement of the tubes in each quadrant, as shown in figure 5 is that of a nine point square with one corner omitted. The bifurcation is dichotomous throughout, the planes of bifurcation being at such angles as to bring the stream by the shortest possible route to points midway between the two points reached

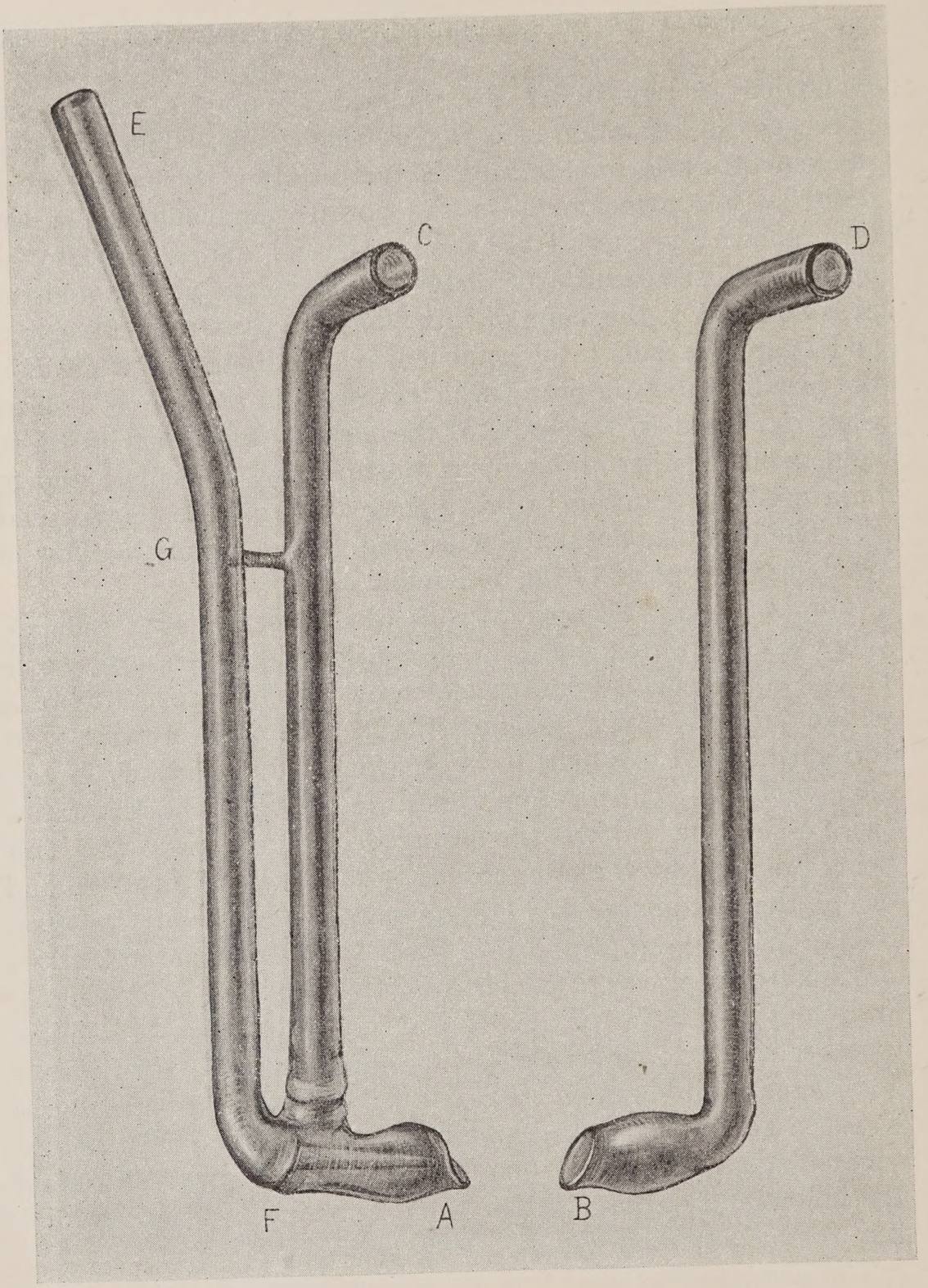

FIG. 4. CANNULAE FOR PORTAL VEIN

A, opening for inflow of blood from distal end of portal vein; *B*, opening for outflow into proximal end of same, or into vena cava; *C*, end connecting with inflow tube of apparatus; *D*, end connecting with outflow tube of same; *E*, side tube for hirudin, fused into cannula at *F* and terminating in inner jet; *G*, glass rod connecting tubes for mutual support.

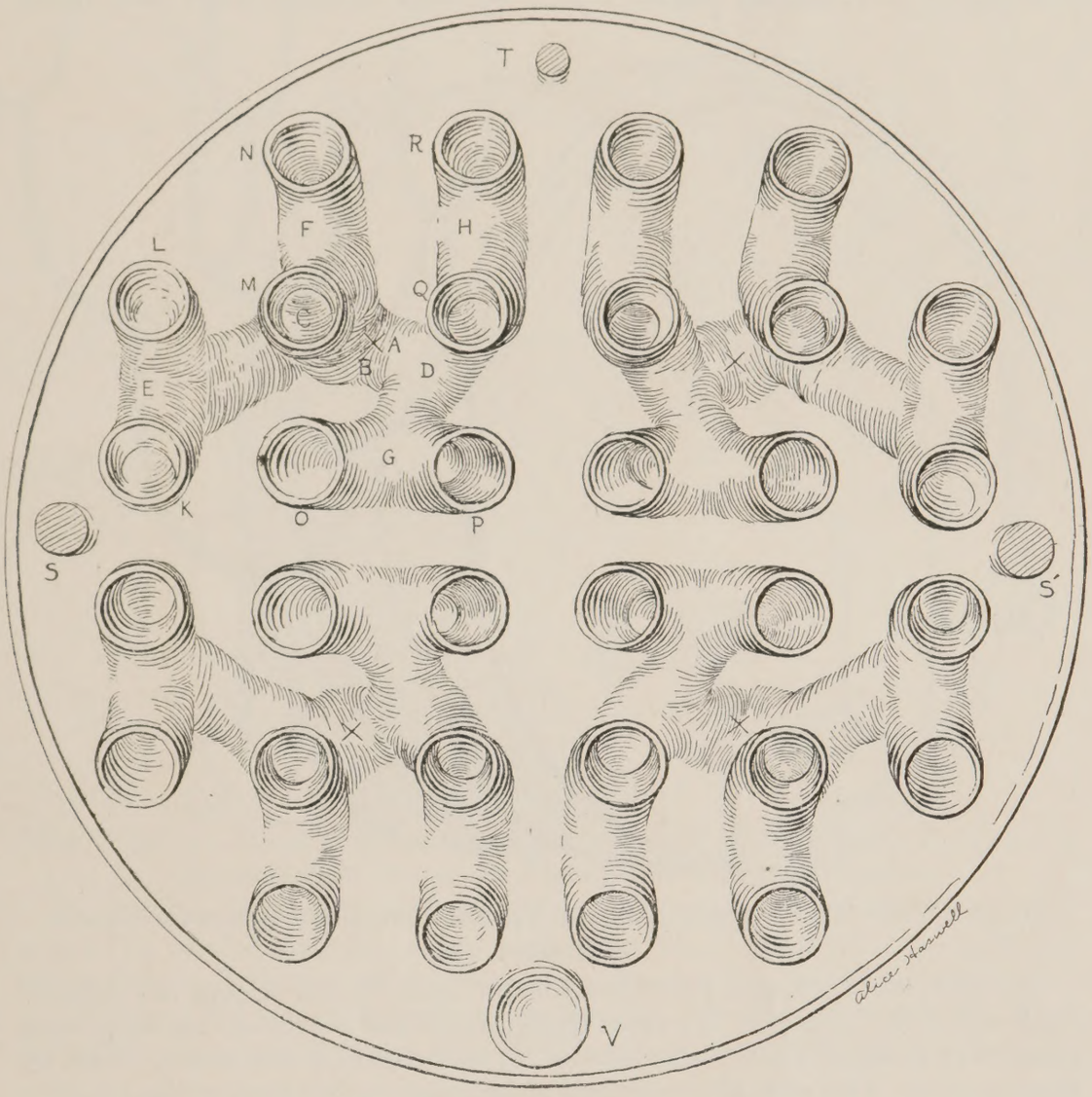

FIG. 5. LARGER APPARATUS WITH THIRTY-TWO TUBES

View of end of apparatus from the interior, showing plan of glass tubes in each quadrant. The two lower quadrants serve for the inflow and outflow of the blood at the proximal end, while the tubes in the two upper quadrants are connected by a U-shaped bend (not shown in figure). At the distal end similar U-shaped connections but in vertical planes connect the upper and lower right hand quadrants, and upper and lower left hand quadrants respectively. The letters *A* to *R* refer to the same points in the course of the tubes as in figures 7 and 8. The crosses show location of inflow and outflow or connecting U-tubes respectively. *S*, *S'*, supporting rods; *T*, thermometer; *V*, tube for inlet and outlet of liquid in jacket.

at the next bifurcation. Thus it will be seen in the plan (fig. 5) that the eight tubes in one quadrant are grouped in four pairs connected by U-shaped bends, three of which are in a vertical and one in a horizontal plane. The further connections are most

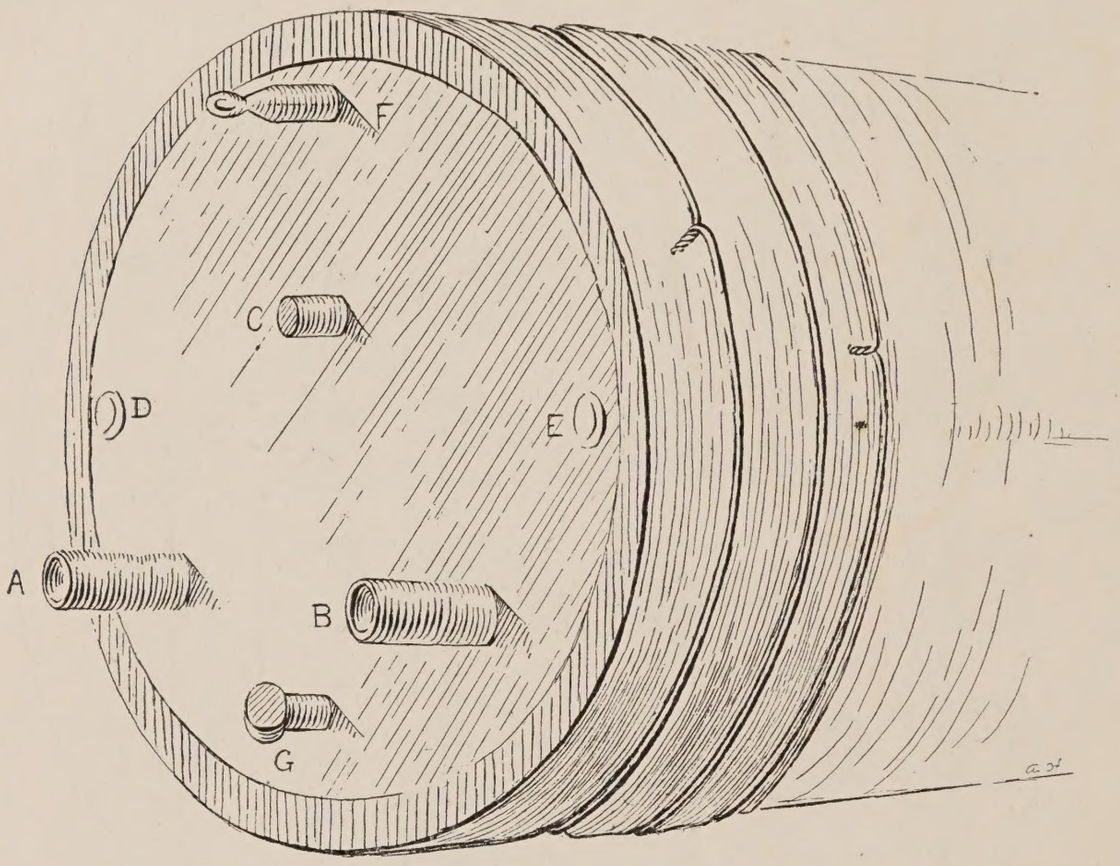

FIG. 6. VIEW OF PROXIMAL END OF THIRTY-TWO-TUBE APPARATUS FROM OUTSIDE

A, inflow tube for blood; *B*, outflow tube for same; *C*, end of glass rod supporting U-tube which connects two upper quadrants; *D* and *E* ends of rods supporting glass tubes at distal end; *F*, thermometer; *G*, spare opening into jacket, closed by glass stopper. This view shows method of fastening rubber stoppers by a rubber sleeve slipped over both stopper and end of jacket and wired on to both, used to prevent leaks and accidental dislocation of stopper.

clearly seen in the diagrammatic sketch figure 7 in which the axis only of each tube is shown, while figure 8 shows the outlines of the corresponding tubes.[7] The letters which refer to the same

[7] The various U-bends are here shown with longer straight stems and broader curves than in the actual apparatus as otherwise the various portions overlap each other in the sketch in a manner too complicated to be intelligible.

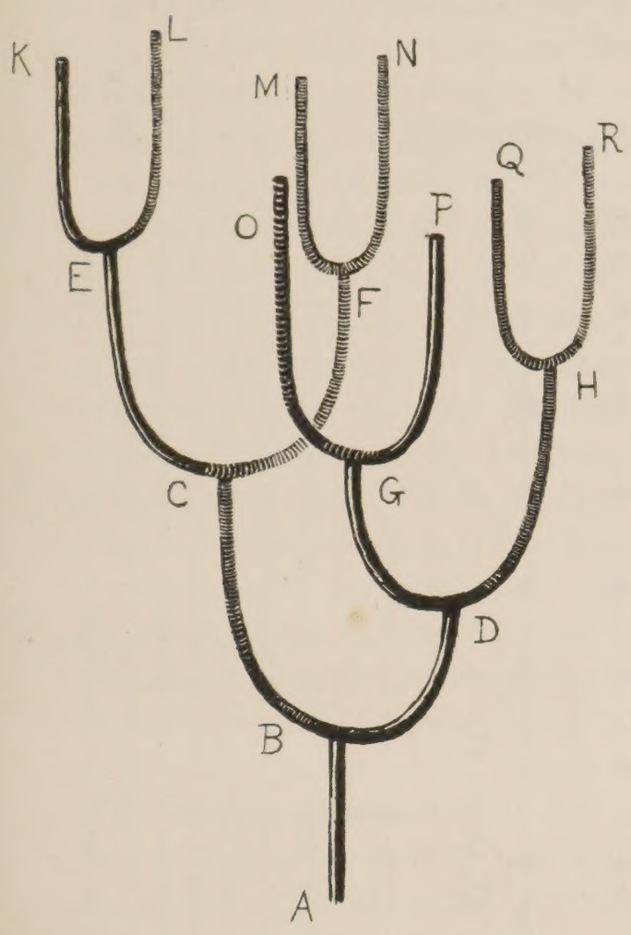

FIG. 7

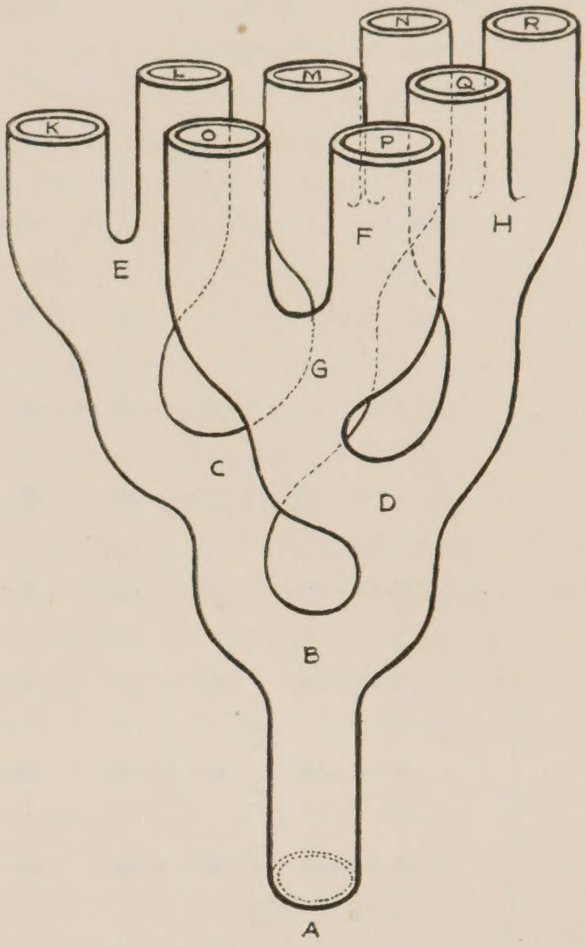

FIG. 8

FIGS. 7 and 8. DIAGRAMMATIC REPRESENTATION OF BRANCHING OF EIGHT-FOLD DISTRIBUTION TUBES OF THIRTY-TWO-TUBE APPARATUS

The letters refer to corresponding points in both figures. The lines in figure 7 refer to the axes of the tubes shown in figure 8. The U-tubes *KEL* and *MFN* are supplied from *C*, the branch *CF* being seen through (i.e., behind) the tube *OG*. The U-tubes *OGP* and *QHR* are supplied from *D*. The three U-tubes *KEL*, *MFN* and *QHR* are in parallel planes while *OGP* is in a plane at right angles.

points in figures 5, 7 and 8 have the following significance: *A* is the inlet tube (indicated by a cross in fig. 5), which bifurcates at *B* leading to the two points *C* and *D*. The tube at *C* supplies the four tubes *K*, *L*, *M* and *N* which are united in two U-tubes the planes of which are parallel, but which are arranged *en échelon* in plan (step shaped, *KLMN*, forming the figure ⅂) while that at *D* supplies the four tubes *O*, *P*, *Q* and *R* grouped in two pairs, the planes of the two U-tubes being at right angles and united

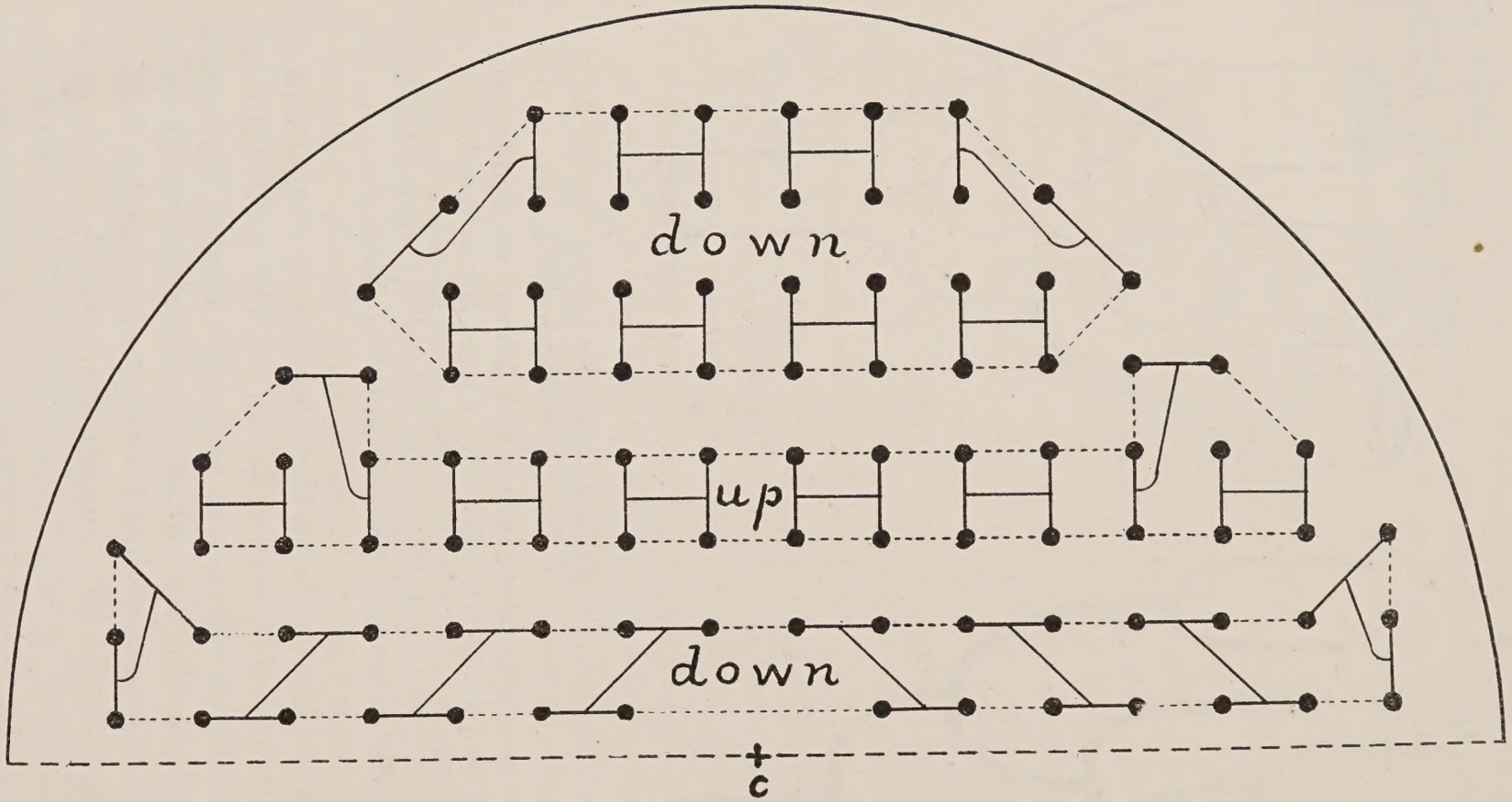

FIG. 9. DIAGRAMMATIC PLAN OF LARGEST APPARATUS, WITH ONE HUNDRED AND NINETY-TWO TUBES

Only one-half is shown, the tube arrangement in the remainder being a symmetrical repetition of that shown. The apparatus consists of six separate sections of thirty-two tubes each through which the blood flows in parallel alternately down and up, three times each way. The position of the tubes is indicated by the heavy dots and their connections in groups of four by the full lines. The further connection of these groups is omitted to avoid confusion. The dotted lines enclose the tubes which are connected together in the several sections. The supporting rods for each section, placed at the ends of the same, and the central rod supporting the whole apparatus, are omitted in this plan.

so that three tubes, *P*, *Q* and *R* are in a row (parallel to the lines *KL* and *MN*), while the fourth tube *O* stands out to one side and comes in line with *M* and *N*.[8] The connections will be easily seen by comparing the two figures.

An apparatus is now being constructed which will have 192 tubes of 6 mm. diameter spaced about 11 mm. apart from center to center contained in a jacket 20 cm. diameter. With celloidin tubes 45 cm. long it is estimated that the internal volume, including connections will be about $2\frac{1}{2}$ to 3 liters, suitable for a calf, a large goat or other animal weighing from 200 pounds upwards. To facilitate the laborious process of tying on the tubes the system is made in six sections each to be provided with its independent supporting framework, the sections to be connected by rubber tubing. This not only makes the tubes much more accessible, there being very few tubes hidden in the interior of a section, but reduces the difficulty caused by leaks. Each section being tested separately but little extra work will be involved in replacing faulty tubes, or retying insecure joints.

The arrangement adopted for the tubes will be evident from figure 9 which represents one-half of the apparatus. Each section contains 32 tubes through which the blood flows in parallel, the branching being on the same dichotomous principle already described. Owing to the complication of the connections, the primary, secondary and tertiary bifurcations are omitted from the plan, which shows only the grouping of the tubes (indicated by heavy dots) in fours, and the outline of the separate sections by means of dotted lines. The blood will flow up and down, in alternate sections, three times each way.

A small form of apparatus, suitable for work on individual organs is represented in figures 10 and 11, the former a diagrammatic view of the glass parts and rubber stopper only, the latter showing two units of this type united together in a manner which will be plain from the legend.

An apparatus is in course of construction which will contain four tubes 120 cm. long of an original diameter of about 33

[8] In other words the four points *E*, *F*, *G* and *H* are at the centers of the four U-tubes *KL*, *MN*, *OP* and *QR* respectively and of these *E* and *F* are connected to *C* while *G* and *H* are connected to *D*.

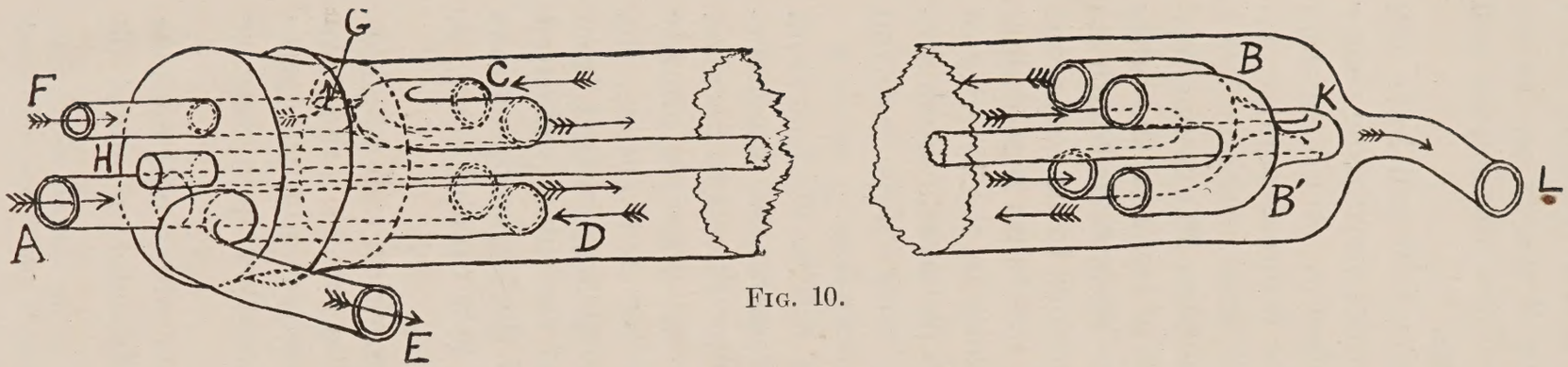

FIG. 10.

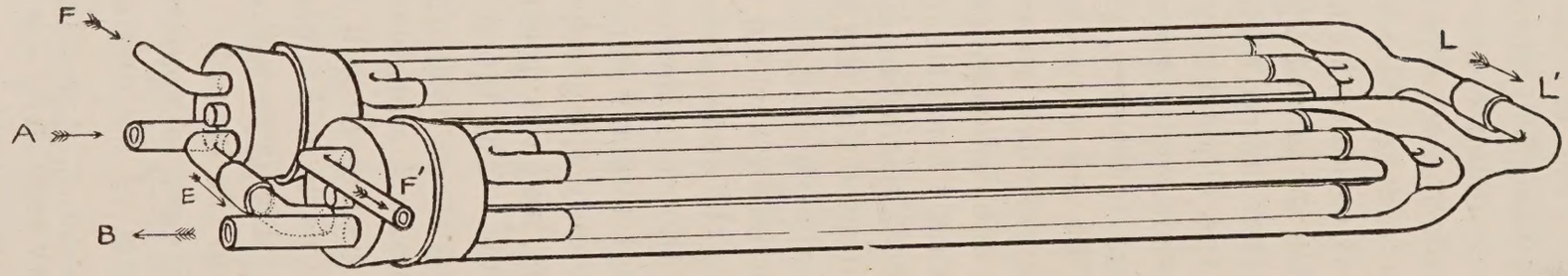

FIG. 11.

FIG. 10. SMALL TYPE OF APPARATUS FOR INDIVIDUAL ORGANS

Has four tubes. Diagrammatic view. *A*, blood inlet; *B*, *B'*, U-tubes for return of blood at distal end; *C*, same at proximal end; *DE*, outlet; *F*, inlet for saline solution which passes through hole in side of tube at *G* into the space outside the diffusion tubes. This tube is closed at the end where it is fused to *C*, which it supports mechanically but with which it does not communicate. *HK*, central rod supporting *B* and *B'*; *L*, outlet for outer fluid. The celloidin tubes are not shown in this view.

FIG. 11. PERSPECTIVE VIEW SHOWING TWO UNITS SIMILAR TO THAT IN FIGURE 10, CONNECTED TOGETHER

A, blood inlet; *B*, outlet; *F*, inlet for saline solution; *F'*, outlet for same. The connections for the blood stream by the tube *E* and for saline solution at *L*,*L'* are made by rubber tubing.

mm. which will be flattened out to a width of 5 cm. and a thickness of about 2 or 3 mm. For this purpose the tubes are to be enclosed in light frameworks of gilded wires which will only cover a small fraction of the diffusing surface while providing supports at numerous points to prevent the tubes bulging under internal pressure. Another type of apparatus has been designed and is also in course of construction in which, without resorting to an excessive number of tubes of inconvenient dimensions, a much larger proportion of diffusing surface to volume is obtained. In this type celloidin tubes carrying salt solution are placed inside similar tubes which carry the blood and which are themselves surrounded by salt solution. We therefore have the annular space between pairs of concentric tubes of celloidin filled with blood. Placing the thickness of this space at 1 mm. and taking into account that two surfaces are exposed, there will be about six to eight times as much surface for a given volume of blood as in the forms described above.

IV. TECHNIQUE OF EXPERIMENTS

The method of attaching the apparatus is always essentially the same although slight variations in technique are necessitated in attacking different problems. The principal factors which may be varied are the site of attachment of the cannulae, the anaesthetic used, the precautions for asepsis, the size of the apparatus and the duration of the experiment.

The experiments fall into two groups according to whether the recovery of the animal is aimed at, or whether the collection of dialyzing substances from the blood is the sole object. The technique in the latter case varies as to whether the material is to be obtained from the general circulation, or from the portal vein, or from the blood leaving an individual organ (e.g., the thyroid or the pancreas) with a view to obtaining specific substances contained in the internal secretion. The technique described in detail in the following paragraphs is that used in collecting material from the general circulation, while specific variations will be considered later.

The animal usually experimented on, up to the present, has been the dog, owing to its convenient size and availability. To insure a rich supply of products of digestion in the blood, a full meal of meat is given 9 to 12 hours previous to the operation, and in some cases a second meal of 100 to 200 grams of meat about three or four hours before commencement. The additional meal, however, is apt to lead to vomiting during the dialysis, for which reason the animal must be closely watched.

Because of its ability to produce prolonged and light anaesthesia with relatively little depression, tertiary trichlorbutyl alcohol (commercially known as "chloretone") has been used by preference. It is administered by stomach-tube about two hours before the operation, using about 0.25 gram per 1 kgm. of body-weight. The powdered crystals are washed down with a little water. This frequently produces the desired anaesthesia without other means, but in some cases a little ether has to be given at certain stages.

Immediately before, or preferably, if sufficient assistance is available, during the operation, the apparatus is washed out several times with distilled water and salt solution, to remove all the thymol[9] which has been used to prevent bacterial growth while the apparatus was not in use.

The tubes are filled by a funnel and rubber tube with a warm solution containing 0.6 gram NaCl in 100 cc.[10] Where the apparatus is of the return flow type, and especially in those cases in which the tubes are arranged to give a flow twice or oftener

[9] Owing to the danger that small particles of thymol may remain adhering to the tubes, and pass into the animal's circulation by solution during the experiment, it is important to use only *dissolved* thymol in the apparatus, and to take care that there is no opportunity for it to crystallize out. The present custom is to put thymol only in the outer solution after each experiment relying on its diffusion through the celloidin to keep the inner liquid aseptic.

[10] Calcium and potassium chloride were included in the solution in the earlier experiments, to approximate closer to the composition of the blood, and the sodium chloride was used in the concentration of 0.9 gram in 100 cc. The calcium chloride was omitted to reduce the chance of clotting and the sodium chloride reduced to restore partially the physiological balance, as certain observations mentioned elsewhere, pointed to the passage of excess of sodium ions into the blood.

in each direction, it is important always to hold the apparatus during the process of filling so that the liquid enters each set of tubes, in turn, *from below* and to reverse the apparatus as soon as the liquid begins to pass round the bend. In this manner the air is completely expelled ahead of the liquid and few or no bubbles remain to be removed by capillary pipettes at the terminal openings.[11] The inlet and outlet tubes, which have been provided with short lengths (about 3 to 4 cm.) of tight-fitting, but not too small rubber tube, are closed by clamps on the rubber, leaving sufficient space beyond the clamps for the introduction of the ends of the cannulae. The outer jacket is then filled with the same saline solution which has been heated to such a point that the temperature of the whole apparatus will be between 38° and 40°C. by the time the blood flow is started. Little heat is required during the course of the experiment with the larger types of apparatus, the heat radiating surface of which is small compared to their heat capacity, and one or two incandescent electric lamps placed beside or under the jacket, with a towel or two over the whole, answers to keep the temperature constant. The arterial and venous cannulae, should be selected before the experiment for suitable size and shape, to avoid delay at the critical moment. They must both be carefully fitted to the individual animal and the particular operation, so that when connected in position they will meet the inflow and outflow tubes of the apparatus in the proper line and allow of insertion through the rubber connecting tubes so far as to make a joint "glass to glass," and at the same time be accurately in line with the blood vessels and avoid all strain on them after adjustment. The caliber of the arterial cannula is selected as large as can be readily inserted in the vessel, while that of the venous cannula may either be taken of maximum size or the caliber may be made somewhat smaller where it is desired to reduce the rate of flow and thereby raise the pressure in the apparatus.[12]

[11] On the same principle, in emptying the apparatus, air is blown in *from above*, reversing at the appropriate moment as in the opposite process.

[12] This has been tried in the hope of obtaining a longer period of dialysis, where the animal was thought to be deficient in resistance or small for the appa-

The usual site selected for the operation has been the neck because of the good blood flow to be obtained from its vessels, but the femoral vessels may be used. The artery is chosen on the side of the animal on which the apparatus is to lie; the vein is usually taken on the opposite side, to avoid crowding at the site of attachment. The carotid artery is exposed low down towards the clavicle, the external jugular at a point about 3 to 5 cm. higher, corresponding to the distance between inflow and outflow tubes of the apparatus.

The incision and the preparation of the vessels is carried out in the usual way, with the only difference that the greatest possible care must be taken to secure all bleeding points by tying, owing to the incoagulability of the blood in the subsequent experiment. After cutting the vessels, the blood remaining above the clamp is removed by pressure and the cannulae, partially filled with weak hirudin solution at once inserted and tied in place: moist sponges may now be placed over the wounds. The recovery of the animal not being contemplated, it is convenient to insure against subsequent oozing of blood from the wound, which in some long experiments has been considerable, by applying at this stage a solution of Monsel's salts or of ferric chloride to all cut surfaces.

The hirudin burette, supported in a suitable stand above the animal, is now filled and attached by rubber tubing to the side tube (*C*, figs. 1, 3) of the arterial cannula, and the solution allowed to flow till the latter is full, all air bubbles being driven out of the open stem.

The apparatus at this stage is best held in the hands by an assistant who should watch carefully that he keeps the inflow and outflow tubes exactly and steadily in the proper position relative to the animal's body, no matter what jerks it may be subjected to in the subsequent process of connecting the tubes: the distal end should be a little lower to bring any chance bubble to the mouth of the tubes. Another assistant fills completely the ends of the cannulae, one at a time, and the ends of the rubber on

ratus; it is doubtful, at the present writing, whether much was gained thereby in duration of the experiment. Of course the initial flow of blood is reduced; the velocity later on is less affected.

the corresponding tubes of the apparatus with saline solution from a pipette while the operator inserts the cannulae. The clamps are then removed from the rubber tubes in question, and the cannulae pushed home "glass to glass" while great care is taken to avoid strains on the blood vessels. The distal end of the apparatus is now raised till slightly higher than the other, and while watching the position relative to the wound, the supporting clamps or blocks are brought into place and made firm. The apparatus, which may conveniently rest on a table adjacent to the operating table, extends in a nearly horizontal direction at right angles to the axis of the animal's body and just above the level of the wound.

The venous clamp is now removed and hirudin solution from the burette allowed to flow into the apparatus, displacing an equal volume of saline solution into the vein. The amount of hirudin required to render the whole blood of the animal completely incoagulable for twelve hours or more has first been estimated from previous tests. It is found advisable to run in from three-quarters to nine-tenths of this amount at once, at this stage, before the admission of blood to the apparatus, as the most dangerous time for the formation of clots, apart from the last stages when the flow slackens and the blood is beginning to stagnate, seems to be in the first hour.

The arterial clamp is now removed whereupon the flow of blood into the system of tubes, displacing and partially mixing with the saline solution, may be readily followed by the eye. The time of complete displacement of saline solution amounting to 30 cc. per kilogram of body weight is about 45 seconds where full caliber cannulae are used and the animal is in good condition, but a satisfactory flow can be obtained with a venous cannula of such caliber that three to four minutes is required for the return of the blood to the vein. This is probably considerably faster than the flow in the last few hours of a long experiment, in which cannulae of maximum size were used.

Information in regard to the coagulability of the blood is easily obtained. As soon as the artificial circulation is established, 2 to 5 cc. of blood are withdrawn from a vein of the hind leg by

puncture with a suitable needle. The condition of the blood is noted after five to six minutes and again at intervals. In the event of immediate clotting more hirudin is allowed to flow into the arterial cannulae and the coagulation test is repeated. Some hours later a test may again be made with 2 cc. of blood from a vein, when it may be found that clotting now occurs within a few minutes although the blood that was drawn earlier in the experiment still remains fluid. Even then there is no immediate danger of blood clots forming in the apparatus. Indeed we have had no serious trouble from this source since our first experiments.

Another procedure pertains to the study of the blood flow in the dialyzer. This consists in clamping off the artery temporarily, allowing hirudin or saline solution to flow in and wash out the blood in the cannula (5 cc.). On releasing the artery clip the rate of flow may be gauged with a fair degree of accuracy. Again, information in regard to the circulation in the apparatus may be obtained by increasing the pressure within the outer jacket just enough to flatten the celloidin tubes somewhat and then noting the speed with which they fill out again when the pressure is removed. Alternate compression and relaxation of the celloidin tubes also serves to agitate the fluid surrounding the tubes thus preventing the formation of layers of saturated solution on their outer surfaces.

The rate and force of the pulse may be easily seen in the arterial cannula at the point where blood and hirudin solution meet; occasional admission of a small quantity of the latter prevents the blood forcing its way too far back in the side tube, and gives a sharp line of demarcation, pulsating with every beat of the heart.

An approximate idea of the condition of the heart and of the state of the general pressure may be obtained in the following way. The stop-cock of the burette containing the hirudin is opened and the burette is raised and lowered until the point is found at which the hirudin solution no longer flows into the dialyzer but remains at a constant level. The height of the column above the heart is now measured and calculated in terms of mercury pressure. In animals that are in good condition the pressure

so measured is often 30 to 35 mm. (Hg) early in the experiment, while later it may be as high as 50 to 60 mm. and towards the end of a long experiment it may reach a low level of 20 to 15 mm. Dogs have sometimes lived as long as two hours when the pressure of the blood in the apparatus obtained by this procedure, was down to 15 mm. At this time the general arterial pressure as measured for example from the femoral artery would naturally have reached a low level and would probably be found to be around 50 mm. (Hg). The dynamical principles which govern the flow of liquids require a fall of pressure wherever, as in this case, a liquid flows rapidly through a constricted place in its channel, the fall being greater or less, according to the velocity; hence the pressure measured in the above manner will always be lower than in the artery, on account of the resistance to flow in the narrow point of the arterial cannula.

Attachment to the portal circulation

It is of interest to study the diffusates from the portal blood and a beginning has been made in this direction by attaching the apparatus to the portal vein (four experiments). Special cannulae, which have already been described (see fig. 4) are needed for these experiments. The portal cannula is filled with leech extract and inserted into the portal vein just below the entrance of the pancreatic vein, while the wide-mouthed venous cannula, also filled with leech extract, is introduced into the vena cava. In one experiment this cannula was introduced into the proximal end of the portal vein, but the difficulties encountered in the insertion of the two large cannulae into the short length of the portal vein available for this purpose were such that we have, for the present at least, selected the inferior vena cava as the carrier of the outflowing blood.

Attachment to the veins of individual organs

This has not yet been attempted by us, though an apparatus suited to this purpose and consisting of separable units has been designed by us and is shown in figures 10 and 11.

Care of the apparatus

At the conclusion of an experiment in which it is not desired to save the animal the fluid is removed from the outer jacket, the apparatus is detached and the contained blood is washed out of the tubes with salt solution which has been saturated with thymol. The jacket is also filled with this solution. The apparatus is then set aside until it is again needed.

In case the experiment has gone on for many hours with an economical use of leech extract it may be found that some of the tubes can not be completely emptied of blood on account of the formation of a few small clots. In this case the blood is washed out of the tubes with salt solution as before. The tubes are then filled with an acid-pepsin solution, the jacket is filled with a 0.6 per cent salt solution acidified to the same degree and the apparatus is them kept at body temperature until the clots are completely digested. The whole apparatus is then washed out thoroughly, filled in the usual way and set aside. If used with care the apparatus can be employed many times without replacing the celloidin tubes. Our apparatus with 32 tubes, figure 5, has remained in perfect condition for a period of eight months. Two tubes only have been replaced during this period, in which the apparatus has been used in about thirty experiments.

V. THE PREPARATION OF THE LEECH EXTRACT

As already stated, an extract obtained from the medicinal leech is employed in our method of vividiffusion to render the blood incoagulable. In consequence of the high price[13] of hirudin, the anticoagulative principle of the leech, we now prepare for ourselves an active extract of this principle, basing our method on the work of Jacobj[14] and his pupils, Franz, Hayashi and Bo-

[13] Hirudin costs $27.50 a gram. We have used more than half a gram in a single experiment of long duration on a large dog. Good medicinal leeches from France can be bought in lots of one hundred or more from cupping barbers at the rate of $6 a hundred. Hynson, Westcott & Co., of Baltimore, inform us that they hope to be able to furnish the best leeches at $20 to $25 a thousand. As a single leech may yield 8 mgm. of active hirudin to extraction with water, the saving that results from making the extract is considerable.

[14] Arch f. Exp. Pathol. u. Pharm. 49, p. 342.

dong,[15] investigators who have given us many valuable data in regard to this singular substance.

We have usually prepared our extract from two hundred leeches though lately we have used a larger number at a time. Only the head and the immediately adjoining segments are used. The head is seized with a pair of forceps and the body is cut through at the tenth or twelfth ring. The head portions are then ground up as fine as possible, with or without the use of sand as the case may be. 150 cc. of distilled water (i.e., 0.75 cc. per head) and enough thymol in substance to ensure asepsis are added and the mixture set aside in the ice-chest for twenty-four hours. The mass is then transferred to a Buchner funnel and filtered under pressure and washed with just enough water to give 150 cc. of filtrate. The filtrate is brought rapidly to a temperature of 82 to 85°C. and acetic acid is added cautiously until the solution is barely acid to litmus. The coagulated proteids are immediately removed on a suction filter and the reaction of the hot filtrate is restored to the neutral point by means of sodium bicarbonate. Jacobj and his pupils have shown that hirudin is sensitive to acid and to heat, losing much of its anticoagulative power when heated for more than a short time at 100°C.

The aqueous extract just described retains its activity for a long time if it be kept on ice and all bacterial change prevented by the addition of sufficient thymol or chloroform. It is made ready for use by the addition of enough sodium chloride in substance to bring it up to a 0.6 per cent solution.

If the leeches are in good condition and have not been starved for too many months[16] a solution thus prepared is highly active. Thus, 0.1 cc. of an extract (150 cc.) obtained from 200 leeches when added to 3 cc. of dog's blood prevented clotting for forty-eight hours. Assuming that rabbit's blood and dog's blood require about the same amount of hirudin to prevent coagulation and knowing from the experiments of Franz that 0.001 gram of commercial hirudin will prevent 5 cc. of blood from coagulating

[15] Arch f. Exp. Pathol. u. Pharm. 52, p. 242.

[16] See work of Jacobj and his pupils already cited.

we may assume that our 150 cc. of first extract contains in the neighborhood of 0.900 gram of hirudin, equivalent to $4\frac{1}{2}$ mgm. per leech. The heads are extracted a second and a third time with distilled water; the second extract is somewhat less active than that just described, having about three-fifths of its coagulative power. The third extract is as a rule poured upon a new lot of freshly ground leech heads, thus serving as the fluid for a first extract of these.

We have lately simplified the method by using the Buchner press. A thousand leech heads are ground up as fine as possible and covered with 1000 cc. of water. Thymol is then added and the mixture is agitated in a shaking machine for a few hours and is then placed in the ice chest over night. In the morning the clear or slightly turbid supernatant fluid is removed by a siphon and the sediment of crushed heads is placed in a piece of toweling and allowed to drip. When the excess of fluid has been removed, the towel with its contents is made into a parcel which is then exposed for some hours to a pressure of 300 kgm. per square centimeter in the Buchner press. When the cake is dry it is removed, allowed to soak in water and again pressed out. This is repeated once more. The combined extracts are treated as before for the removal of proteids.

In this way a thousand leech heads may be made to yield 1210 cc. of an aqueous extract of such strength that 0.15 cc. will keep 5 cc. of dog's blood from clotting for twenty-five hours. Calculated against commercial hirudin each cubic centimeter of the extract should contain about 6 mgm. of hirudin. In addition to the above extract there were obtained 192 cc. of "washings" each cubic centimeter of which contained 2.5 mgm. hirudin as estimated by the clotting tests.

Inasmuch as this extract contains small quantities of constituents of the leech's body other than hirudin, such as mucin, and no doubt also small amounts of amino-bodies of non-proteid character, it was of interest to learn the amino-N content of the extract. This was done by using Van Slyke's micro-apparatus and it was found that each cubic centimeter of the extract contained 0.12 mgm. of aliphatic amino-nitrogen. Ten milligrams

of commercial hirudin in the form of dry scales analysed by the same method[17] gave 0.15 mgm. amino-N. Hirudin is stated to be a secondary albumose and has been shown by Bodong[18] to be but slightly diffusible, as practically none dialyzes through parchment in nine hours. Inasmuch as our extract contains 6 mgm. of hirudin in each cubic centimeter it is evident that a good part of its nitrogen may be considered to be present in this non-diffusible form. A consideration of the analytical data just given shows clearly that we are introducing into the body of the dog with our leech extract only little more amino-nitrogen than would be the case if we used the commercial article. We have confined our attention for the present to a consideration of the amino-nitrogen of the extract as compared with that of the commercial article for the reason that we are now engaged in the study of the amino-bodies found in the dialysate of our apparatus. If the necessity arises we shall later differentiate more carefully between the various nitrogenous constituents of the extract.

VI. EMPLOYMENT OF THE APPARATUS NOT DETRIMENTAL TO LIFE

As far as our present studies have enabled us to determine, the apparatus, even under the present conditions, may be attached to an animal and the blood be allowed to course through it for several hours without inducing any untoward effects or injuring the animal in any way.

This was established in two experiments in which the operation was performed under surgical asepsis, the one being carried on for two, the other for three hours. In each case the apparatus was attached to the left femoral artery and the right femoral vein. A solution made up of 0.6 per cent NaCl and 0.1 per cent KCl was used for filling both the dialysing tubes and the jacket. The amount of hirudin solution used, the intervals between the tappings of fluid from the dialyser, anaesthesia (ether only) and other factors approximated as nearly as possible to the conditions of experiments previously described. Both ani-

[17] Jour. Biol. Chem., xvi, p. 121. 1913.
[18] Loc. cit.

mals made a quick recovery and in the weeks following during which they were kept under observation nothing abnormal was noted, on the contrary as is usual in such cases, the animals improved in condition and took on weight because of the good care they received.

It is difficult to see how the experiment can be detrimental to life when carried on for only three or four hours. In experiments of long duration the removal of substances needful to the economy, such as sugar, amino-compounds, ferments, etc, can be prevented by the simple expedient of putting into the fluid that surrounds the dialysing tubes an amount of each of these substances just equal to that contained in the blood. We base our belief in the non-harmfulness of the procedure when the proper safeguards are taken not only on our experience with animals that have recovered from the operation but also upon the following considerations:

1. The hirudin solution is not detrimental tolife. Bodong[19] has shown that the cautious injection of hirudin into a vein of the rabbit even in amounts as large as 51 to 75 mgm. pro kilogram of body weight has no influence upon the circulation or the respiration, and is in no other way harmful to the animal. Our considerable experience with hirudin leads us to believe that this is true also for dogs.

2. The celloidin membranes do not give up to the blood anything as, e.g., a trace of nitrocellulose, which in any way affects the blood pressure. Assurance on this point was obtained in the following way. Four hundred cubic centimeters of blood was taken from a dog and allowed to mix with the quantity of hirudin solution necessary to prevent clotting. The blood was then divided into four portions A, A′, B and B′.

A = 100 cc. To this was added 16 tubes of celloidin, 24 cm. long and 8 mm. in diameter, which had been cut up into small pieces.

A′ = 100 cc. Control blood, nothing added except the hirudin.

A and A′ were kept at body temperature for sixteen hours.

[19] Arch. f. exp. Pathol. u. Pharmakol., 52. 254.

B = 100 cc. To this was added celloidin in pieces and equal in quantity to that used in A.

B′ = 100 cc. Control blood, contained no foreign substance except hirudin.

B and B′ were kept on ice for sixteen hours.

The pieces of celloidin were removed from A and B and the four lots of blood were examined in respect to their action on the blood pressure and respiration of a small dog weighing 7 kgm. Both A and A′ had assumed a venous color from the long exposure in the thermostat at 37.5°C. and in the hope of correcting this condition oxygen was allowed to bubble through them for a few minutes prior to the injection.

When injected intravenously neither A nor B had the slightest influence on the blood pressure as measured by the mercurial manometer; there was no difference observable between their action and that of their controls, A′ and B′. Indeed, none of these specimens of blood had any more action on the blood pressure than a similar quantity of 0.8 per cent salt solution.

3. The arterial pressure is lowered because of the considerable amount of blood that is withdrawn for circulation through the dialysing apparatus, but the fall in pressure is not so great as to influence seriously any of the vital functions, such as the circulation or the respiration, provided that the volume of blood withdrawn by the dialyser is not too great.

As repeatedly stated in this paper, the low state of the circulation toward the end of a very long experiment (ten or more hours) in which not more than 30 cc. of blood per kilogram of the animal's weight is contained in the dialysing apparatus, is to be referred to the combined influence of several factors, such as the loss of stimulating constituents,[20] the binding down of an animal in one position for many hours and the long-continued anaesthesia.

The following protocol of an experiment shows clearly that an apparatus which holds even as much as 36 cc. per kilogram

[20] We have found that the alcohol soluble substances eliminated in the dialyser raise the arterial pressure of a normal dog when injected intravenously.

of body weight may, for some hours at least, be attached to the carotid artery and to the jugular vein by cannulae of such wide calibre as to allow of a rapid and free flow through the apparatus without depressing the blood pressure to a point incompatible with life.

Experiment of March 15, 1913. Dog of 5.8 kgm. Had been kept on meat and water for five days. Used a modified Ringer's solution containing only 0.9 per cent NaCl and 0.1 per cent KCl[21] for the inside of the dialysing tubes and for filling the outer jacket. The dialyser had 16 celloidin tubes 20 cm. in length and 8 mm. in diameter; these with their connecting tubes held 210 cc. The apparatus was attached to the left carotid artery and right jugular vein. The mercurial manometer for registering the blood pressure was attached to the left femoral artery. A cannula was inserted into the right femoral vein. Chloretone (Trichlor-tertiary-butyl alcohol) given by mouth was the anaesthetic used.

12.20 Arterial pressure, 120 mm.

12.25 0.3 gram of hirudin in 75 cc. of saline solution.

12.27 The arterial clip was removed and blood allowed to flow through the apparatus.

12.28′. 10″. Arterial pressure, 64 mm.

12.35 Arterial pressure, 60 mm. Injected 1 gram of sodium salicylate into the femoral vein. This caused at first no alteration in the mean arterial pressure, although inducing considerable vascular relaxation.

2.00 p.m. Changed the fluid in the outer jacket; arterial pressure 60 mm.

2.50 Arterial pressure 48 mm. Left vagus cut; arterial cannula torn out while trying to free the right vagus. Experiment terminated.

In the above experiments too much blood was allowed to course through the apparatus and the arterial blood pressure was lower than we care to have it in experiments of this kind. The arterial pressure here approaches that seen in surgical shock.

[21] Owing to the fact that solutions of this high salt content cause oedema and possibly also injure the heart because of the potassium chloride, we now use only a 0.55 to 0.60 per cent NaCl solution.

That the conditions of the above experiment were less favorable to vitality than usual, was shown by the striking and unique condition that developed at autopsy. It was found that rigor mortis had set in very quickly. The heart was so firmly contracted that the cavity of the ventricle was practically obliterated. About 100 cc. of bloody fluid was found in the abdominal cavity and much also in the pleural cavity. The lungs were firm and oedematous and much fluid was contained in the bronchi and trachea. The intestines also were filled with fluid. The kidneys were soft and oedematous and fluid dripped freely from their cut surfaces.

On the prevention of oedema in experiments with the dialysing apparatus

It was only in our earlier experiments that we were hampered by the appearance of oedema in the lungs and elsewhere. Respiratory difficulties arose and at autopsy it was found that a general oedema had developed. In all of these experiments small animals were connected with an apparatus, as in the experiment cited, which withdrew too much blood from the body. We are of the opinion that the high salt content (0.9 per cent NaCl) of the solutions used both within and without the celloidin tubes at this time, together with the low blood pressure, sufficiently account for the appearance of the oedema. In all experiments since about the middle of March, 1913, we have used sodium chloride solutions of 0.55 per cent to 0.6 per cent with or without the addition of potassium chloride and since then we have seen no development of oedema. The pleural and the abdominal cavities have remained dry and the lungs have shown no trace of oedema.

VII. QUANTITATIVE DATA RELATING TO THE ELIMINATION OF SALICYLIC ACID BY VIVIDIFFUSION

We have selected salicylic acid with which to test the eliminating power of our apparatus for the reasons (1) that quantitative estimations of this drug are easily made by colorimetric

methods; (2) that it is a substance of average diffusibility, and (3) that the time required for its complete elimination by the natural eliminating organ, the kidney, is known.

The following protocol gives the details of an experiment on the excretion of salicylic acid by an apparatus having 32 celloidin tubes each 40 cm. in length and 8 mm. diameter which was attached to the carotid artery and the jugular vein of a dog weighing 22.5 kgm. One gram of sodium salicylate was injected slowly into the femoral vein and at first hourly, and later two-hourly analyses were made of the diffusate, this being tapped off and replaced by fresh saline. Each diffusate was shaken three times with ether and after removal of the ether the amount of salicylic acid and of salicyluric acids present in the residue was determined colorimetrically, using ferric chloride in the usual way. In this experiment, during the first seven hours of which there was a good flow of blood through the apparatus, we recovered by this method 165 mgm. of salicylic acid in this time, which represents an output in the seven hours of 191 mgm. of sodium salicylate, or 19.1 per cent of the total amount given. As some loss is unavoidable in the many manipulations involved in the separate hourly estimations it is safe to assume that the actual output of salicylates in the apparatus is somewhat higher than that found.

It is interesting to learn that the urine in the bladder of the dogs used in experiments of this kind is usually entirely free from salicylic acid. The total amount of urine in the bladder at the end of this experiment was less than 20 cc. We have always observed that little or no urine is secreted by the kidneys in experiments of this kind. This fact is not surprising in view of the combined effects of the anaesthetic, the somewhat lowered arterial pressure and the outpouring of diffusible substances through the artificial kidney. This last factor is surely of importance, as the natural kidney hereby loses the chief stimulus to activity.

It may be of interest to compare the rate of elimination of salicylic acid by our apparatus even in its present very imperfect state, with the rate of elimination by the kidneys of an animal that is not depressed by anaesthetics or operative proceed-

ures. A small bitch (hound) weighing 7 kgm. was given 1 gram of sodium salicylate intravenously, the urine of the first six hours thereafter was removed by the catheter, acidulated with sulphuric acid and exhausted with acetic ether and then with ethyl ether until free of the acid. By this means we recovered 151.3. mgm. of free salicylic acid determined colorimetrically, = 175.5 mgm. of sodium salicylate, or 17.5 per cent of the amount injected. It will thus be seen that the animal eliminated in the natural way in six hours 1.6 per cent less than was removed from the blood by the dialyser in seven hours (19.1 per cent) in the experiment referred to above. These data show that the apparatus can already compete with the kidneys on favorable terms, at least during the early hours of the dialysis.

In a later experiment the bitch just referred to received one gram of sodium salicylate by the mouth. Absorption from the gastric tract seems to have been somewhat delayed as only 43.8 mgm. of salicylic acid (=50.7 mgm. sodium salicylate) were recovered from the urine in the first six hours while 378 mgm. (=438 mgm. sodium salicylate) were recovered in the next eighteen hours. This gives an average hourly rate of excretion for this period of 24.3 mgm. a rate which is somewhat higher than could be obtained with our diffusion apparatus in its present imperfect form for an equally long period of time.

Salicyluric acid. That salicyluric acid is also eliminated in the apparatus was shown by using Mosso's[22] method. The residues left behind after removing the ether from the extracts referred to above were dissolved in water and the united solutions were evaporated to dryness on the water bath, water was again added and again driven off, the process being repeated four times in all. The salicylic acid is then supposed (Mosso) to have been driven off completely and if a color reaction is still obtained with ferric chloride this is declared to be due to the presence of salicyluric acid. Such a reaction was obtained by us but no attempt was made to estimate the amount of salicyluric acid eliminated by our apparatus during the entire experiment for the reason that some of the extract was lost in an accident.

[22] Arch. f. exp. Pathol. u. Pharmakol., xxvi, p. 267.

Experiment A. Protocol of experiment to determine the rate of elimination of salicylic acid which has been referred to in the preceding pages. May 14, 1913. Dog weighing 22 kgm.

12.51 Apparatus connected with left carotid and right external jugular vein. Hirudin solution allowed to flow into the apparatus and displace the contained saline solution into the animals system.

12.53 Arterial clip removed and blood allowed to flow into the apparatus and thence back into the body.

1.03 1 gram of sodium salicylate dissolved in 25 cc. of water injected very slowly into the femoral vein.

2.03 Dialysate removed from the apparatus and fresh saline (0.6 per cent NaCl plus 0.1 per cent KCl) solution introduced. Salicylic acid recovered from this first hour dialysate = 48.7 mgm.

3.11 Dialysate removed. Salicylic acid recovered (second hour dialysate) = 25.4 mgm.

4.18 Dialysate removed. Salicylic acid recovered (third hour dialysate) = 23.2 mgm.

5.30 Dialysate removed. Salicylic acid recovered (fourth hour dialysate) = 22.0 mgm.

7.38 Dialysate removed. Salicylic acid recovered (fifth and sixth hours) = 30.2 mgm.

8.50 Dialysate removed. Salicylic acid recovered (seventh hour dialysate) = 15.1 mgm.

10.00 Dialysate removed. Salicylic acid recovered (eighth hour dialysate) = 9.3 mgm.

12.10 Dialysate removed and combined with that from the next period for analysis.

2.20 Dialysate removed. Salicylic acid recovered (ninth to twelfth hour) = 27.8 mgm.

2.30 Animal very weak. Pulse rate 134. Respiratory rate 31.

4.15 Dialysate removed. Salicylic acid recovered (thirteenth and fourteenth hours) = 5.8 mgm.

4.20 Animal moribund. 4 a.m., pulse rate 122; respiration labored,
5.00 rate 28. 4.35, pulse rate, 107; respiratory rate, 31. Salicylic acid recovered = 2.3 mgm.

5.10 Animal dead.

An examination of the above protocol shows that the output of salicylic acid began to drop rather sharply at the end of the

seventh hour. During the last four hours the circulation was weak and this sufficiently accounts for the diminished output. Even under these conditions, however, the total amount of salicylate recovered amounts to 24.35 per cent of that injected. We need hardly call attention to the importance of being able to remove so considerable a proportion of a lethal dose of a poison.

Experiment B. May 2, 1913. Dog weighing 11.3 kgm. A small apparatus used, (16 tubes) holding 260 cc. of blood. Apparatus attached to left carotid artery and right external jugular vein.

12.45 Apparatus attached and hirudin solution (0.4 per cent) allowed to flow slowly into the apparatus the clip on the jugular vein being removed. About 40 cc. of hirudin solution used, more being injected later.

1.02 Arterial clip removed.

2.25 Dialysate removed and fresh solution introduced into the apparatus.

2.30 0.99 gram sodium salicylate in 20 cc. of water injected slowly into left femoral vein.

3.40 Dialysate removed. Salicylic acid recovered (first hour) = 24.12 mgm.

4.55 Dialysate removed. Salicylic acid recovered (second hour) = 15.22 mgm.

5.55 Dialysate removed. Salicylic acid recovered (third hour) = 13.23 mgm.

6.55 Dialysate removed. Salicylic acid recovered (fourth hour) = 11.40 mgm.

8.20 Dialysate removed. Salicylic acid recovered (fifth hour) = 10.50 mgm.

9.05 Dialysate removed. Salicylic acid recovered (sixth period forty-five minutes) = 9.63 mgm.

It will be seen that the total amount of salicylic acid recovered by dialysis in the above experiment in five and three-quarter hours was 84.10 mgm. = 97.5 mgm. sodium salicylate. The average hourly output was, therefore, 17 mgm., which again does not compare so very unfavorably with rate of excretion by the kidney for a similar period when the drug is given by the mouth. That the output of salicylic acid by the dialyser is considerably less

in this experiment than in experiment A is fully explained by the smaller dialysing surface of the apparatus.

As already stated, the present form of apparatus is susceptible of great improvement. A form possessing much larger dialyzing surface in proportion to the volume of blood passing through it will naturally remove the diffusible constituents of the blood as well as foreign substances that have been introduced much more rapidly than the types that have been described. In using more effective dialysers for the removal of foreign or toxic substances from the animal organism, it will be necessary to introduce inside the outer jacket either serum or a fluid that closely approximates to serum in composition in order that the body may not be robbed of necessary constituents, as sugar, while it is giving up a particular substance which it is desired to remove. The results in regard to salicylic acid that have been reported above, as well as other data that have been gathered by us in experiments on dogs and on rabbits encourage us to proceed further with the problem of the removal of toxic substances from the blood by means of a diffusion apparatus.

VIII. SUBSTANCES THAT ARE KNOWN TO BE ELIMINATED FROM THE BLOOD BY THE APPARATUS

It must be evident as stated in the introduction that our apparatus makes no distinction among the diffusible constituents of the blood allowing these to pass into the outer fluid at a rate that is presumably proportional to the coefficients of diffusion. When the fluid within the outer jacket consists of a 0.55 per cent or 0.6 per cent solution of sodium chloride, as in the experiments described, it is found that many of the well known constituents of the blood and urine soon accumulate in it. Thus sugar and urea (the latter isolated and identified by its melting point and other properties) appear in large quantities. Phosphates and diastase are also found.

Ethyl sulphide $(C_2H_5)_2S$, which is readily evolved from dog's urine on the addition of lime water or other alkali may be obtained in like manner from the concentrated diffusate, showing

that the mother substance of this malodorous compound is freely eliminated by our apparatus. Of more interest is the fact that we now have at our disposal a method for accumulating the non-proteid nitrogenous constituents of the blood other than urea in any desired amount, the quantity possible to be obtained depending on the size of animal used, the dialysing surface of the apparatus and the number of experiments performed.

Already, in June of this year we had fractionally distilled several grams of the esters of the amino-acids obtained by Fischer's method. Much of our time this autumn has been devoted to accumulating material. The method followed is, concisely, as follows. The dialysate is concentrated on the water bath at a low temperature, neutralizing with acid as required, until a large part of the sodium chloride has crystallized out: this is filtered off, the filtrate diluted somewhat, made acid (5 per cent) with HSO_4, and precipitated with phosphotungstic acid. The precipitate is decomposed with baryta, while the filtrate is freed from sulphuric and phosphotungstic acids by the same reagent and excess of baryta removed. The liquid is then carefully brought to the neutral point and evaporated in vacuo. The greater portion of the large mass of sodium chloride is separated from the amino-bodies by methyl alcohol. Quantitative determinations have shown the presence of at least 5 grams of amino-nitrogen, exclusive of urea which we remove by the action of urease,[23] in the liquid accumulated during the autumn in this manner. If we assume a proportion of about 10 per cent of nitrogen in the amino form as a rough average for the amino-bodies present we should have about 50 grams of these substances in our hands at the present time. We also plan to collect similar material from the blood of herbivora by means of the large dialyser shown in figure 9. The further chemical separation of these amino bodies as well as of the substances precipitated by phosphotungstic acid will be reported in a later paper.

[23] E. K. Marshall, Jour. Biol. Chem., xiv, 283, 1913, and xv, 487, 1913. Van Slyke, Jour. Biol. Chem., xvi, 128, 1913.

IX. SUMMARY

1. A method has been devised by which diffusible constituents may be removed from the blood of a living animal, which does not involve any procedure prejudicial to life.

2. Two animals have made rapid and complete recovery after being subjected to the procedure for two and three hours respectively.

3. The method has been shown to be available for collecting from the blood, under the ordinary conditions of physiological experimentation, substances present only in small amount at one time.

4. Several types of apparatus have been constructed adapted to various purposes, and full details as to methods of construction are given.

5. Experience has been accumulated on the use of hirudin, and the procedure adopted for the economical preparation of solutions of this active principle from leech heads is given in detail.

6. As an organ of elimination of abnormal substances (e.g., poisons), quantitative results obtained with salicylic acid show that the apparatus in its present form compares not unfavorably with the kidney. The direction of improvement is indicated and experiments in this direction are in progress.

7. Data as to the effect of the procedure on blood pressure are given. It is shown that general oedema in striking degree may result from neglect of certain precautions.

8. Material has been collected in large quantity for the study of the non-proteid amino-bodies present in the blood. The chemical separation of these bodies is in progress and only preliminary results are here given.

9. Directions in which the method may be utilized, both for the study of problems in physiological chemistry, and as a promising therapeutic agent, have been indicated.

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